

電界誘起型マイクロバブルメスによる細胞への試薬導入



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混相流体に対応可能な遺伝子導入方法とは

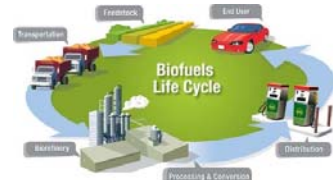
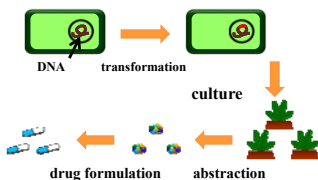
Background

Transformation of plant cell

Classification of gene transfer by physical techniques

Pharmaceutical technology

Physiologic study for bio fuel



High-viscosity material to be injected

mRNA

5 kg/mol

Artificial gene

200 kg/mol

	Electroporation	Sonoporation	Micro-injection	Bubble knife
Resolution	High	Low	High	High
Target	Animal cell only	Animal cell & plant cell	Animal cell & plant cell	Animal cell & plant cell
Ability of Transportation	Low	High	Low	High
Handling of micro-volume	Low	Low	High	High

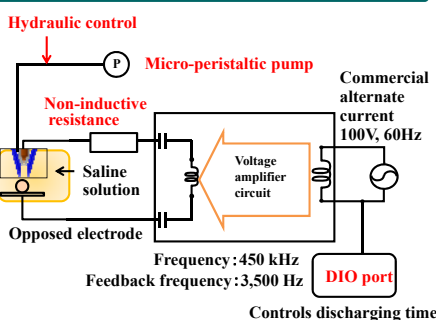
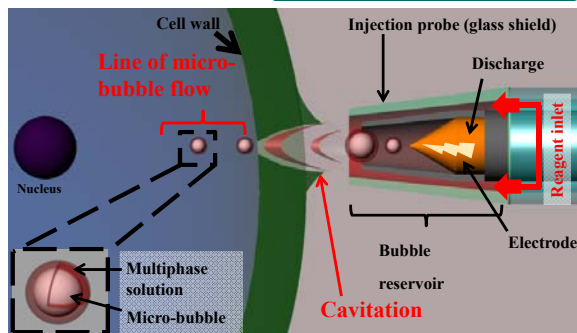
Required condition of gene transfer into plant cell

- ✓ **High perforation ability** to pierce cell wall of plant cell
- ✓ **High transportation ability** for transportation of high-viscosity and high-molecular weight subject to be injected
- ✓ **Handling of micro-volume**

Concept

Concept of transportation using bubble knife

Overview of electrical circuit

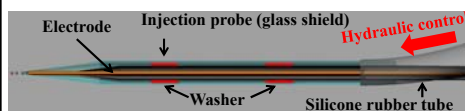


Fabrication

Process flow of bubble knife

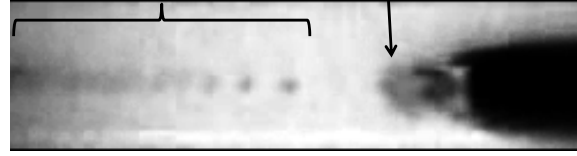
1. Insulation of Cu micro-wire into glass tube
2. Disconnect by thermal glass puller
3. Insulation of micro-electrode into injection probe

Schematic overall view of bubble knife



Underwater discharging of bubble knife

Line of micro-bubble flow Cavitation



Concept of multiphase flow injection

Perforation of cell wall by using cavitation
 Young's modulus of cell wall is GPa order, but cavitation is enable to perforation of metal
Transportation by using micro-bubble flow and adsorption force of micro-bubble

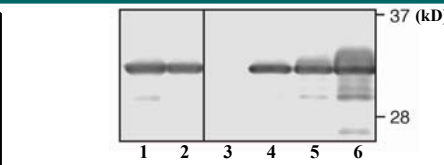
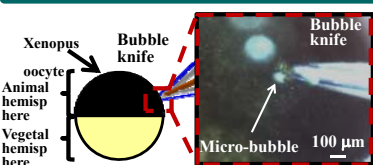
Experiment

Biocompatibility and chemical action to reagent

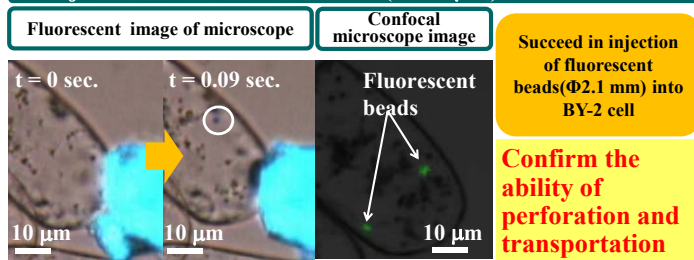
Perforation ability and transportation ability

Injection of mRNA into xenopus

Injection of fluorescent beads (Φ2.1 μm) into BY-2 cell



Confirmed translation of GST-FLAG by western blotting
 Succeeded in injection of mRNA and translation and injection volume was 100~200 pL



Succeed in injection of fluorescent beads(Φ2.1 mm) into BY-2 cell
 Confirm the ability of perforation and transportation

Conclusions

1. We succeed in injection of Fluorescent beads into BY-2 cell by using perforation ability of cavitation and transportation ability of micro-bubble flow and adsorption force.
2. For evaluation of biocompatibility and chemical action to reagent, we injected mRNA into Xenopus and confirmed translation of GST-FLAG was successfully operated.

Reference

Hiroki Kuriki, "Simultaneous local ablation and injection into cell by electrically-induced micro-bubble knife", Proceedings of Robomech2013, 1P1-O06, 2013

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